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the alkali was neutralized directly to different points. Twenty-four hours' action on glyceryl triacetate gave the following results:

H^+ ion concentrations	10^{-4}	10^{-6}	10^{-8}	10^{-10}
Activities	0.15	0.10	1.17	1.47

Two similar experiments gave the following results:

H^+ ion concentrations	10^{-7}	10^{-8}
Activities	0.91	1.68

The action was therefore greater in slightly alkaline solution.

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[1] These PROCEEDINGS, **1**, 136 (1915).
[2] *J. Amer. Chem. Soc.* (1911).

THE EXCRETION OF ACIDS BY ROOTS

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Whether roots excrete acid, other than carbonic, has long been a matter of controversy. The problem is important not only because acids dissolve plant food from the soil, but also because it involves the fundamental questions of the reaction of protoplasm and of the mechanism of secretion.

The problem has been greatly complicated by the failure of many investigators to distinguish between the effects of dead and of living cells.

Becquerel early pointed out that all seedling roots when laid on moist neutral litmus paper possess the property of giving it a lasting red color, which he believed was due to excretion of acetic acid. Boussingault thought that the acid might be lactic, while other investigators left the nature of the acid undetermined. The alkali salts of formic acid have been reported by Czapek[1] in the culture solution of *Lepidium* and *Hordeum* seedlings. The formic acid was considered as coming not from the root hairs but from the sloughing off of root cap cells and their secondary decomposition. The drops occurring on root hairs in a moist atmosphere were found to give no acid reaction. The solvent[2] action of plant roots has been considered to be due not alone to the respired CO_2 but also to organic acids because phosphorite (which can be taken up to some degree by plant roots), requires a strong acid to dissolve it. Kunze[3] believes that there is no free mineral acid in the root excretion of higher plants, but that the acid reaction is due to organic acids and that the quantity of detectable acids is very small, lying below the limit of sensitivity of litmus. Czapek[4] concluded that roots gave off K, Ca, Mg, HCl, H_2SO_4 and H_3PO_4 to water. Stocklasa and Ernest[5] have been unable to find K or H_3PO_4 in root excretions. They found that CO_2 is the only gaseous product in root excretions, with a possibility of H. No free mineral acids were considered to be present in root excretions, and organic acid excretion was considered to occur only when the oxygen percentage was low. Breazeale and LeClerc[6] state that the roots of wheat seedlings in distilled water excrete acids other than CO_2.

In some of the investigations mentioned above no control of distilled water without seedlings was run, and the amount of alkali used to titrate a boiled sample of distilled water, in which seedlings had grown, appears to be approximately the amount required to cause coloration of the indicator in such a volume of neutral solution. Seeds were in some cases in contact with the solution and might have given off acid from dead cells. In some cases it appears certain that dead cells of the roots affected the results. The use of carbon black by some investigators introduces an unknown factor which may alter the results.

The experiments of the writer were made upon early sweet corn by soaking the seed for several hours and then germinating it on moist filter paper in porcelain pans covered by inverted pans. When the roots were about one to one and one-half inches long, the seedlings were placed in the water cultures. Glass tumblers were steamed and thoroughly cleansed, and the outside covered with paper to prevent the growth of algae. Dental napkins, in which one rather large and numerous small holes were cut, were used to cover the tumblers after the distilled water was run in.[7] The cloth covers were drawn tight by the use of string and never were allowed to come into contact with the solution. The larger hole was used to run in more water as evaporation proceeded while in the small holes the seedling roots were placed. Covers of various kinds were used because the water evaporated too rapidly from the tumbler through the cloth. The same kind of cover, with a thin coat of high grade paraffine, prevented much evaporation since the large corn seed covered the hole through which the root had been thrust. Also no tying of the cover was necessary; simply folding down the overhanging edges of the cover about the sides of the tumbler sufficed. Great care was taken to use only roots which had no dead cells (except those of the root cap).

Experiment 1. Twenty-five seedlings were grown in each tumbler in 190 cc. of distilled water. Controls were set up similar in every respect (except that they contained no seedlings) and having the holes in the covers partially covered so as to obtain about equal amounts of evaporation in all the tumblers. A series of phosphate buffer solutions, of known hydrogen ion concentration, and checked by the use of the gas chain, were made up. Phenolsulphonephthalein (12 mgm. per 100 cc.) was used as indicator (3 drops to 10 cc. of solution). The indicator was added to a series of tubes containing the various buffer solutions whose PH^+ value was known.[8] After the plants had grown for 8 days, 10 cc. of the water from each tumbler was run into a tube of the same diameter as those containing the buffer solutions and after adding 3 drops of the indicator, the CO_2 was expelled by a stream of hydrogen (washed free from impurities). The hydrogen was allowed to run through each tube for one-half to three-quarters of an hour to be sure of expelling all the CO_2.

TABLE 1

Results after CO_2 *was expelled*

Water from tumblers containing roots	Water from controls without plants
1. P_H+ 7.38	1. P_H+ 7.38
2. " 7.38	2. " 7.38
3. " 7.38	3. " 7.38

Experiment 2. Seedlings with vigorous roots and with apparently no dead cells, were grown for 5 days in culture. The distilled water was different from that in Experiment 1.

TABLE 2

Results after CO_2 *was expelled*

Water from tumblers containing roots	Water from controls without plants
The water from 7 tumblers each P_H+ 7.16	The water from 6 controls each P_H+ 7.16

A similar experiment in glass tumblers was run for 19 days. During that time the cultures received no attention whatsoever. Analysis showed no increase of acidity, even though the roots were full of brown spots.

The use of glass tumblers might be objectionable because of the possible dissolving of the glass, resulting in the possible neutralization of any excreted acid, but in that event the controls ought to indicate an increased alkalinity.

The experiment was then repeated, using large quartz dishes instead of glass tumblers. The covers were made of large sheets of filter paper impregnated and coated on both sides with a thin layer of high grade paraffine, melting point 60–62°C. Holes of the required size were cut out of the cover with a sharp cork borer. The cover was laid on the dish after the distilled water had been added, and care was taken not to let the cover touch the water at any time. The two quartz dishes each contained 650 cc., and two smaller quartz dishes each contained 50 cc. of pure distilled water. One of the large quartz dishes had 65 corn seedlings growing in it while the other had 77 seedlings. The seeds never came in contact with the water. The seedlings were grown in the culture for 7 days. The water in the dishes was made up daily to the same volume as at the start and the evaporation was kept approximately the same in each dish by covering or uncovering the extra holes in the covers. At the end of 7 days the corn roots were about 6 inches long and apparently free from brown spots. The water was then tested as in the other experiments, the CO_2 being expelled from the samples by running hydrogen (free from impurities) through them for 45 minutes each.

TABLE 3

Results after CO_2 *was expelled*

Quartz dishes containing seedlings	Quartz dishes containing only water
65 seedlings: Water P_H+ 8.04	No seedlings: Water P_H+ 7.88
77 seedlings: Water P_H+ 7.88	No seedlings: Water P_H+ 7.88

The two covers bearing the seedlings were then lifted from the top of the quartz dishes and by means of a sharp razor blade (that had been thoroughly cleansed) the roots were cut off a short distance below the seed and were allowed to fall into the dishes in which they had been growing. The tops and the attached seeds were discarded. The paraffin covers were put on the dishes and the holes in the covers were closed by laying several layers of filter paper over them. After two weeks, numerous brown spots appeared on the roots, and many of the roots became soft and gelatinous toward the tip. After 4 weeks from the time that the experiment was first begun, the water in which the roots were decomposing appeared brownish, but possessed no disagreeable odor. Analysis of the water was made as described before.

TABLE 4

Results after CO_2 *was expelled*

Quartz dishes without roots: control	Quartz dishes containing cut-off roots
Control quartz dish......Water P_H^+ 7.88	Quartz dish with decaying roots..............Water P_H+ 8.04
Control quartz dish......Water P_H^+ 7.88	Quartz dish with decaying roots..............Water P_H+ 8.04
	Two new samples of water were taken to verify the results:
	Quartz dish with decaying roots..............Water P_H+ 8.04
	Quartz dish with decaying roots..............Water P_H+ 8.04

After the experiment was ended, it was found that the mass of roots was so soft as to be easily compressible by the fingers into a small ball. The solutions were tested with litmus paper prior to taking out the roots from the dishes. Experience with litmus paper has brough out the fact that it may require some time before the paper indicates the reaction of a solution which is close to the neutral point. The water, that contained the decaying roots, gave no evidence of being acid to blue litmus paper even when the water contained CO_2.

The corn seedlings had been used because of the convenience in making cultures, and because of the opportunity to choose only healthy seedlings having no brown spots on the roots. Whenever any brown spots appeared on the roots before the time for the first analysis the culture was discarded.

Having found that no acid, other than CO_2 was excreted from the roots of corn, experiments were made upon wheat seedlings. Porcelain pans (with no spots on the inside of the pan) were used. The pans were about twelve inches in diameter at the top and were quite shallow. The same volume of water was run into each pan. Bronze screening was dipped a few moments into nitric acid and then washed a long time in running water. If any acid had remained adhering to the wire, we should expect to find acid in certain of the pans at the end of the experiment. The screening was dried and then dipped into hot high grade paraffine until the screening was coated. The paraffined screens were placed as covers upon the pans of water but were not allowed to come in contact with the water.

Wheat seeds were soaked over a half day in water and were sown in a uniform layer over four of the screens, the other two screened pans of water being used as controls. In two of the four seeded screens, the screens were purposely pushed down until they touched the water and in these two pans the seeds also were partially in contact with the water. Pans were inverted over the seed until they had germinated and their roots had entered the distilled water. The seedlings were then exposed to the light and the control pans were always treated in the same manner.

The cultures were allowed to grow for four weeks without attention of any kind. The plants had grown about 6–8 inches in height in about a week after germination and then ceased to grow. At the end of the experiment the roots in no case were coiled up but grew straight downward. The roots of the seedlings had softened and were well decayed after four weeks. Analysis of the water was made as in the other experiments.

TABLE 5

Results after CO_2 *was expelled*

Control (screen not touching the distilled water)	Water P_H+ 7.6
Control (screen not touching the distilled water)	Water P_H+ 7.6
Pan with seedlings (screen or seed not touching the distilled water)	Water P_H+ 8.0
Pan with seedlings (screen or seed not touching the distilled water)	Water P_H+ 8.0
Pan with seedlings (screen and seed touching the distilled water)	Water P_H+ 8.3
Pan with seedlings (screen and seed touching the distilled water)	Water P_H+ 8.2

The experiments with corn seedlings indicate that no acid other than CO_2 were excreted by the roots. The data for corn seedlings shows an exceedingly small increase in the alkalinity of the distilled water when the roots were permitted to decompose in the water. The increase in alkalinity of the one culture, of 65 seedlings of corn in quartz at the end of 7 days, probably indicates the presence of some dead cells that were not apparent. Distilled water, into which only the roots of wheat seedlings extended, showed a very slight increase in alkalinity when the roots had decayed, but when the screen, germinated and ungerminated seeds, and roots were in the water, the increase in alkalinity was slightly greater.

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[1] Czapek, F., *Biochemie der Pflanzen,* **2,** 872, 1905.

[2] T. Pfeiffer u. E. Blanck, *Landw. Versuchstat, Berlin,* **77,** 217 (1912).

[3] Kunze, G., Jahrb. wiss. Bot., **42,** 357 (1906).

[4] Czapek, F., Zur Lehre von den Wurzelausscheidungen, *Ibid.,* **29,** 321.

[5] Stocklasa, J., and Ernest, A., *Ibid.,* **46,** 73 (1908).

[6] Breazeale, J. F., and LeClerc, J. A., *Bull. U. S. Dept. Agric. Div. Chem.,* No. 149 (1912). See also Meurer, R., *Jahrb. wiss. Bot.,* **46,** 503 (1909), and Johnson, H. V., *Amer. J. Bot.,* **2,** 250 (1915).

[7] The water was distilled from a glass still (which had been used for some months) without the use of cork or rubber. The first and last parts of the distillate were discarded.

[8] This method was adopted because the indicator must be accurately calibrated by buffer solutions in order to obtain results of any value. Phenolsulphonephthalein is very useful for such investigations.

SPECTROGRAPHIC OBSERVATIONS OF RELATIVE MOTIONS IN THE PLANETARY NEBULAE

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In these PROCEEDINGS, 2, 129 (1916), we described certain rotation phenomena of the planetary nebula N. G. C. 7009 (R. A. = 20 h. 58 m.), as observed by means of the Mills spectrograph attached to the 36-inch refracting telescope. In the past nine months, as opportunity offered, we have extended this line of investigation to others of the